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Morphological and physiological characteristics of *Sclerotium rolfsii* causing stem rot or rhizome rot disease in tuberose *(Polianthes tuberosa)*

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ABSTRACT

Tuberose is the most popular ornamental bulbous perennial plant cultivated throughout the world especially in India for the cut and loose flower trade. But this tuberose is most affected by stem rot (Sclerotium rolfsii Sacc.), one of the most destructive and widespread soil-borne diseases. In Tamil Nadu, tuberose cultivation is highly interrupted by this pathogen. The survey was conducted in different tuberose growing districts of Tamil Nadu and eight different isolates of pathogenic fungi were obtained viz., SR1 to SR8. Among them, SR1 produced the white cottony mycelium (9.0cm), reddish-brown colour sclerotia with maximum sclerotial size (1.69mm) and weight (180 mg100^{-1} sclerotial bodies).Under pot culture conditions, the various isolates show classic stem rot symptoms. Based on the different degrees of virulence, SR1 was identified as the most virulent isolate (88.00 %). The PDA medium showed maximum radial growth of the fungus (9.00cm) and a similar result was also obtained in potato dextrose broth (7.30g). Among the seven different carbon sources, starch was recorded as the best source (8.29cm) followed by dextrose (8.18cm). Among the six different nitrogen sources tested, sodium nitrate (8.77cm) recorded maximum growth followed by potassium nitrate (5.38cm). The maximum growth of the pathogen was recorded at 30^{0}C (9.00cm) and pH 7.0 (8.92cm).

***Keywords*:** Morphological character, *Sclerotium rolfsii*, stem rot, tuberose

Floriculture is one of the age-old-practice in our nation. Flowers represent purity, beauty, serenity, love, admiration, innocence, passion, and many other emotions. Tuberose (*Polianthes tuberosa* L.) is a commercially important ornamental plant cultivated in the world as well in India for the cut and loose flower trade. It belongs to *Amaryllidaceae* family. The flowers are attractive with sweet fragrances (Sadhu and Bose, 1973). Tuberose is affected by various fungal, bacterial and viral diseases. Among the different fungal diseases stem rot disease is caused by *Sclerotium rolfsii* Sacc. (Teleomorph: *Athelium rolfsii*) which is one of the major problems in tuberose growing areas (Das, 1961). This pathogen affects production and crop cultivation. It can destroy tuberose fields. It is a necrotrophic soil-borne fungal plant pathogen having a wide host range of over 500 plant species in 100 families mostly affecting cucurbits, crucifers and legumes (Ferreira and Boley, 1992). The pathogen causes different types of diseases such as damping of seedling, collar rot, stem rot, foot rot, rhizome rot, crown rot, sclerotium wilt and blight (Tang *et al.,* 2015). *Sclerotium rolfsii* affected various crops in the world and caused an annual loss of about 30,000 million dollars (Chaurasia *et al.,* 2014). At the time of infection, the saprophytic pathogen produced more quantity of oxalic acid and cell wall degrading enzyme that kills the host epidermal cell before penetration (Higgins, 1927). *S. rolfsii* can survive in a wide range of environmental conditions. An optimum temperature of about 27-35^{0}C is required for optimum growth and sclerotial production. But hyphal growth occurs at 8-40^{0}C. Water saturated soil is favorable for sclerotial production and hyphal growth. The optimum temperature required for host infection and penetration was 27-30^{0}C(Tu and Kimbrough, 1978). Specific symptom of the disease is the withering of infected plants and leaves followed by yellowing and finally completely dried. The fungus mainly affects the underground parts of the plant specifically roots and suckers, then infection spreads upward through the tuber and collar portion of infected plant. Finally affected roots and tubers get decayed. The affected collar portion contains thick cottony growth of the fungus with a large number of visible mustard-like sclerotia. In severe infection, the losses due to stem rot disease might go up to 50-60 per cent (Kakade *et al.,* 2017). In Tamil Nadu, the losses due to stem rot range from 20-40 per cent (Theradimani *et al.,* 2018). The present experimental study was

Short Communication

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focused on the morphological (colony growth, sclerotial bodies, colour, shape, size and weight) and physiological characteristics (different media, carbon, nitrogen sources, temperature and pH) of various isolates of *Sclerotium rolfsii* under *in-vitro* conditions.

Flaccidity and drooping leaves were symptoms of the illness. The leaves turned yellow to brown and began to wilt. The fungus mostly damages the roots, spreading upward via the tuber and collar portions of the plants. Both the tubers and the roots are decaying. On the decaying stem, petioles at the soil level, thick white cottony growth of the fungal mycelium became evident and a greater number of mustard-like sclerotia where produced.

The stem rot infected tissues of tuberose plants were collected and the infected tissues were used for the isolation of plant-pathogen in Laminar-air-flow chamber under aseptic conditions by "tissue segmentation method"(Rangaswami and Mahadevan, 1998). The pathogen *S.rolfsii* was identified based on the production of fluffy mycelium and sclerotia (Barnett and Hunter, 1972).The infected tissues were washed with distilled water and cut by using a sterilized scalpel from the infected portion along with healthy tissues (3 mm size) and surface sterilized for 30 seconds with 0.1 per cent sodium hypochlorite solution. After washing with the sterile distill water, the infected plant tissues were placed in Petri plate and incubated at 27±1^{0}C.

The mixing of sand and maize powder at 19:1 ratio was done and it was moistened with sterile distilled water @400 ml kg^{-1} which was packed in a clean polythene bag. The bags were autoclaved for two hours for two consecutive days at 1.4 kg cm^{-2} pressure. Then the medium was inoculated with a 9 mm pathogen culture disc under aseptic condition and medium was incubated at room temperature for fifteen days.

The sieving was done by passing the sand, soil followed by farmyard manure through 2 mm mesh and separately sterilized then mixed at the ratio of 1: 1: 0.5. After mixing it was transferred into the sterilized pot up to 30 cm height and then the mass multiplied pathogen inoculum was inoculated at the rate of 5 % of the total weight of the potting soil. In each pot, three tuberose suckers were planted and three replications were maintained along with control.

Eight isolates of pathogen were grown on PDA medium. Using a sterilized cork borer 9 mm pathogen disc was taken from pure pathogen culture plate and placed on the sterilized Petri plate containing 20 ml of PDA medium and the plates were incubated at room temperature. After 48 hours of the incubation period, the hyphal growth of the pathogen was observed. Then the mycelial growth, mycelium colour, colony morphology, sclerotial production, sclerotial colour and sclerotial size were recorded.

Ten different media which included PDA, Carrot dextrose, V8 juice, Cornmeal, Czapek's dox, Malt extract, Yeast extract, Nash Synder, Oatmeal agar, and Tuberose flower extract agar were prepared for this study and autoclaved for 20 min at 15 lbs pressure.

Ten different liquid media *viz*., Potato dextrose, Carrot dextrose, V8 juice, Cornmeal, Czapek's Dox, Malt extract, Yeast extract, Nash Synder, Oat's meal, and Tuberose flower extract broth were prepared and 100 ml of each broth were transferred into clean conical flasks separately and sterilized at 15 lbs pressure for 20 min. Then, each broth was inoculated with a 9 mm actively growing pathogen culture disc under aseptic condition and the medium were incubated at 28±2ºC for 7 days. After the incubation period, using Whatman No.1 filter paper the pathogen hyphal mat was filtered and dried. The mycelial dry weight was taken along with the filter paper and the actual dry weight of the pathogen hyphae was obtained by subtracting the weight of filter paper.

Carbon source in Czapek's dox agar medium was replaced with various carbon sources such as dextrose, starch, mannitol, cellulose, galactose, sucrose, fructose and lactose were prepared separately and autoclaved. The cool medium was poured into the Petri plates and allowed to solidify. The 9 mm pathogen culture disc was placed at the centre of the Petri plate and the plates were incubated at 28±2ºC.

The nitrogen source in Czapek's dox agar medium was replaced with different nitrogen sources such as potassium nitrate, calcium nitrate, sodium nitrate, ammonium nitrate, peptone and urea which were prepared separately and autoclaved. The cool medium was poured into Petri plates and allowed to solidify. The 9 mm pathogen culture disc was placed at the centre of the Petri plate and were incubated at 28±2ºC for three days.

The impact of various temperatures on the hyphal development of pathogen was studied using PDA medium. The plates were incubated at various range of temperatures viz., 18ºC, 20ºC, 25ºC, 28ºC, 30ºC, and 35ºC for three days. Observations were taken three days after inoculation.

The influence of various pH ranges of the medium on the growth of pathogen was studied using PDA. The medium was prepared with various pH levels *viz*., 5.0 to 9.0 by using 0.1 N Hydrogen chloride or 0.1 N Sodium

Table 1: Morphological and cultural characteristics of various isolates of *S. rolfsii* under *in vitro* condition

S.No	Isolate	Mycelial growth (mm)	Colony morphology	Number of sclerotial/plate	Colour of sclerotia	Shape of sclerotia	Distribution over media	Duration for sclerotial production (day)	Size of the sclerotial bodies (mm)	Test weight (mg) (100 Sclerotial bodies)
1	SR1	90.00	White fluffy mycelium	200	Dark brown	Oval shape	Periphery	9th	1.69	180
2	SR2	86.34	Cottony profuse mycelium	185	Light brown	Round/ Irregular	All over	10th	1.55	163
3	SR3	87.24	Cottony white mycelium	199	Light black	Round/Spherical	All over	13th	1.19	170
4	SR4	88.00	Profused white cottony mycelium	198	Dark brown	Irregular	Periphery	15th	1.10	178
5	SR5	85.30	Dull white mycelial growth	189	Dark brown	Round/ Irregular	Periphery	13th	1.15	170
6	SR6	88.33	Puffy cottony white mycelium	100	Brown regular	Oval	All over	15th	1.39	70
7	SR7	87.36	Fluffy pure white coloured mycelium	90	Brown rough	Semi oval shape	Periphery	12th	1.40	80
8	SR8	89.00	White fluffy mycelium	160	Dark brown	Irregular	All over	15th	1.06	140

Means of three replication – DMRT (P-0.05)

Table 2: Impact of various solid media on the mycelial growth of *S. rolfsii under In-vitro* condition

Treatments	Different solid media	Colony morphology	Mycelial growth (cm)*	Days to sclerotial formation	Distribution over media	Colour of sclerotia	Shape of sclerotia	Size of sclerotia (mm)*	No. per plate
T_1	Potato dextrose agar	Fluffy white mycelium	9.00[a]	9	All over	Dark brown	Round	1.20 - 1.25	180
T_2	Carrot dextrose agar	Cottony profuse mycelium	8.85[b]	13	All over	Dark brown	Round	1.20	167
T_3	V8- Juice agar	Cottony white mycelium	6.44[f]	16	Periphery	Light brown / Brown	Round	0.96 - 1.00	160
T_4	Cornmeal agar	Dull white mycelium	8.80[b]	14	All over	Light brown	Round	1.13 - 1.20	140
T_5	Czapek's dox agar	Dull white mycelium	7.90[d]	15	Periphery	Brown	Round	1.06 - 1.10	80
T_6	Malt extract agar	Puffy white mycelium	7.60[e]	15	Periphery	Dark brown	Round	0.90 - 1.00	96
T_7	Yeast extract agar	White mycelium	8.66[c]	16	All over	Brown	Round	1.15 - 1.20	60
T_8	Nash Synder agar	Dull white mycelium	2.03[h]	-*	-	-	-	-	-
T_9	Oat's meal agar	Cottony white mycelium	8.74b[c]	15	All over	Dark brown	Round	1.15 - 1.18	120
T_{10}	Tuberose flower extract	White fluffy growth of mycelium	6.07[g]	17	Periphery	Brown	Round	0.97 - 1.00	50
	SEm(±)				**0.06**				
	LSD (0.05)				**0.14**				

*Means of three replications

Table 3: Impact of different liquid media on the hyphal growth of *S. rolfsii*

Treatments	Broth	Mycelial dry weight(g)*
T_1	Potato dextrose broth	7.30[a]
T_2	Carrot dextrose broth	6.26[b]
T_3	V8- juice broth	5.66[de]
T_4	Cornmeal broth	5.96[cd]
T_5	Czapek's dox broth	5.37[e]
T_6	Malt extract broth	5.71[d]
T_7	Yeast extract broth	6.03[bc]
T_8	Nash synder broth	1.35[f]
T_9	Oat's broth	6.10[bc]
T_{10}	Tuberose flower extract broth	6.08[bc]
	SEm(±)	**0.14**
	LSD(0.05)	**0.29**

Table 4: Impact of carbon sources on the growth and development of pathogen

Treatments	Carbon sources (solid media)	Mycelial growth(cm)*
T_1	Sucrose	4.80[c]
T_2	Starch	8.29[a]
T_3	Fructose	7.10[b]
T_4	Maltose	7.40[b]
T_5	Dextrose	8.18[a]
T_6	Galactose	4.10[d]
T_7	Lactose	2.00[e]
	SEm(±)	**0.06**
	LSD(0.05)	**0.13**

Table 5: Impact of various nitrogen sources on the growth and development of pathogen

Treatments	Nitrogen sources (solid media)	Mycelial growth(cm)*
T_1	Potassium nitrate	5.38[b]
T_2	Sodium nitrate	8.77[a]
T_3	Ammonium nitrate	3.90[d]
T_4	Urea	1.27[e]
T_5	Peptone	4.47[c]
T_6	Ammonium chloride	3.97[d]
	SEm(±)	**0.05**
	LSD(0.05)	**0.10**

Table 6: Impactr of different pH levels on the growth and development of pathogen

Treatments	pH level	Mycelial growth(cm)*
T_1	5.0	5.55[c]
T_2	6.0	7.67[b]
T_3	7.0	8.92[a]
T_4	8.0	7.89[b]
T_5	9.0	7.85[b]
	SEm(±)	**0.11**
	LSD(0.05)	**0.24**

Table 7: Impact of different temperature levels on the growth and development of pathogen

T. No	Temperature (^{0}C)	Mycelial growth(cm)*
T_1	18	3.10^f
T_2	20	4.40^e
T_3	25	6.19^d
T_4	28	7.65^c
T_5	30	9.00^a
T_6	35	8.77^b
	SEm(±)	**0.09**
	LSD(0.05)	**0.19**

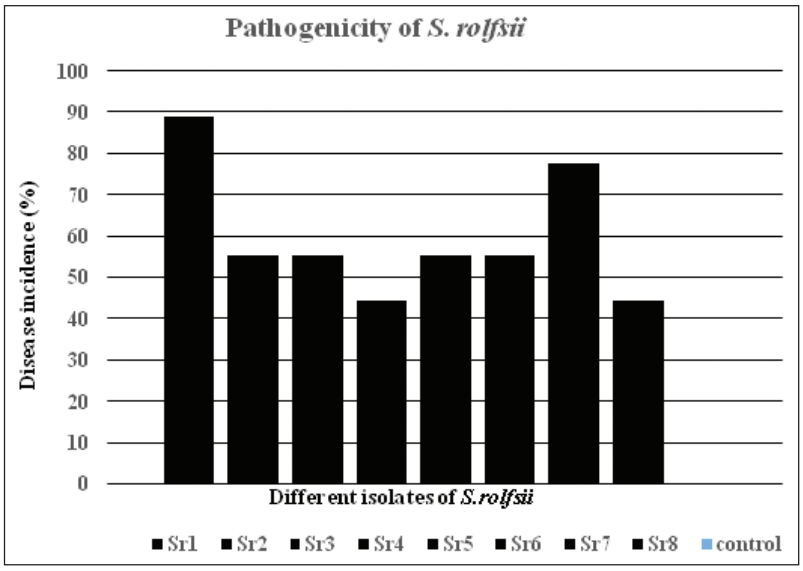

Fig. 1: Pathogenicity test of various isolates of *S. rolfsii* under pot culture condition

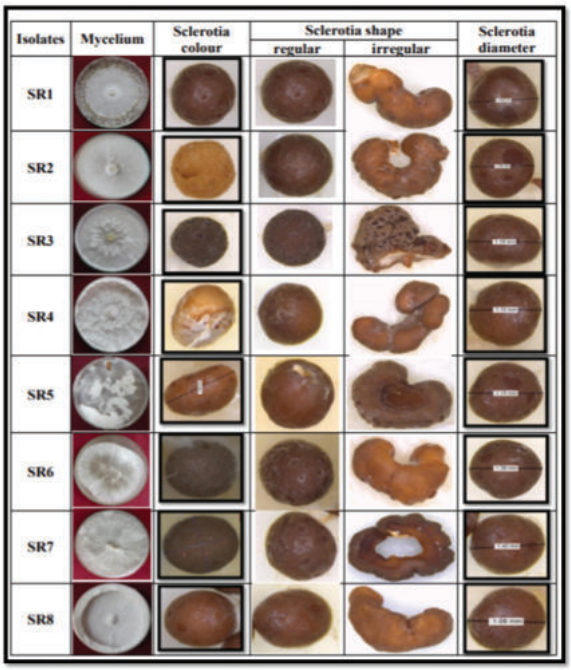

Fig. 2: Morphological and cultural characteristics of various isolates of *Sclerotium rolfsii* under *in vitro* condition

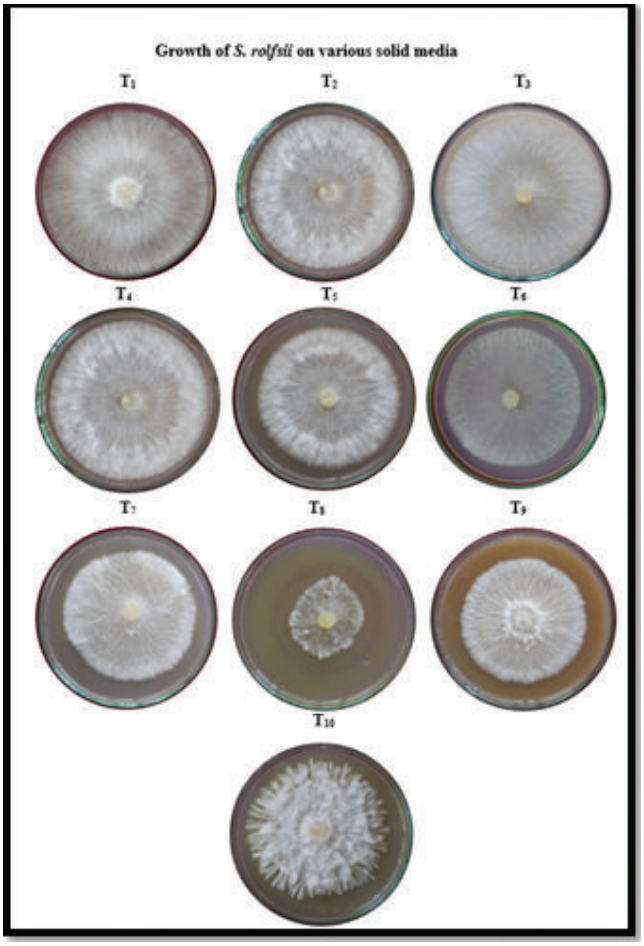

Fig. 3: Growth of *Sclerotium* on different solid media

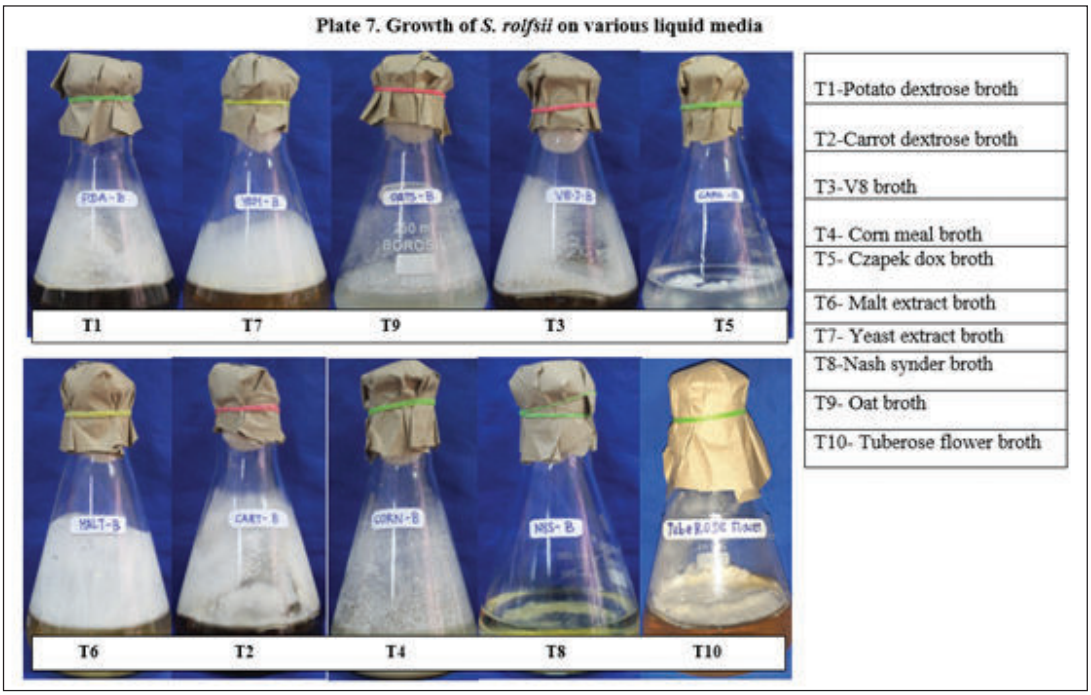

Fig. 4: Impact of various liquid media on the hyphal growth of *S. rolfsii*

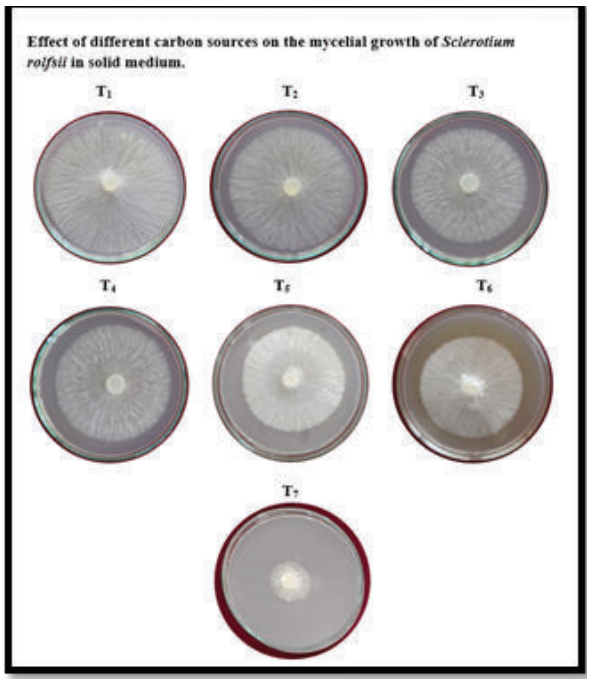

Fig. 5: Effect of different carbon sources on the mycelial growth of *S. rolfsii*

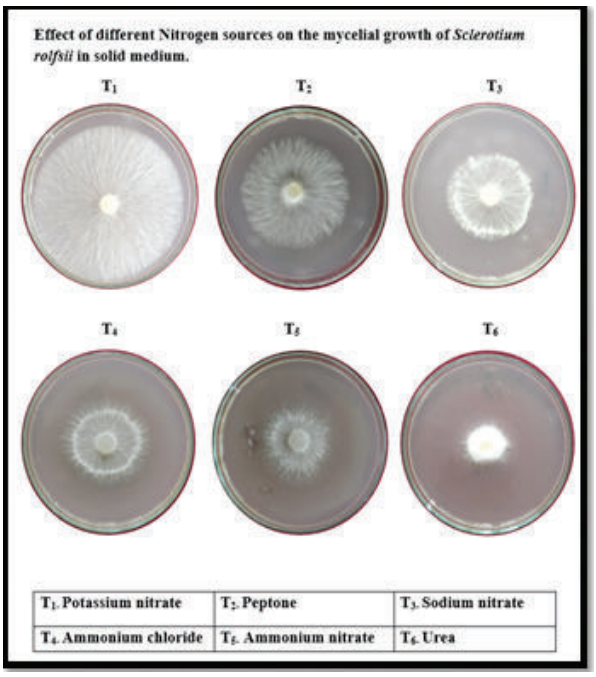

Fig. 6: Effect of different nitrogen sources on the mycelial growth of *S. rolfsii*

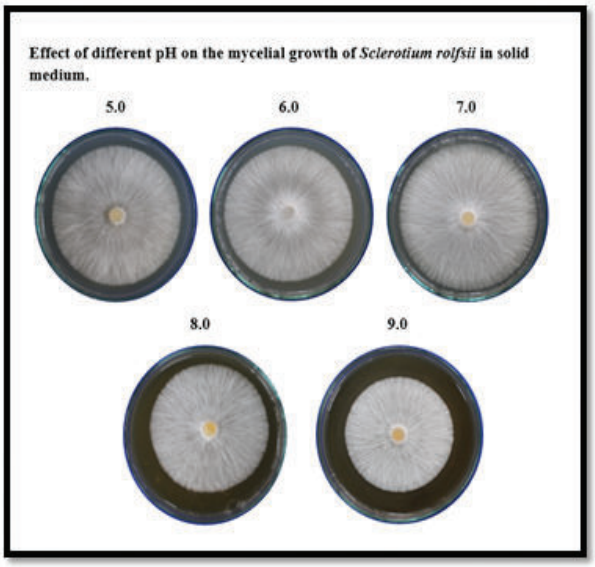

Fig. 7: Effect of different ranges of pH levels on the hyphal growth of *S. rolfsii*

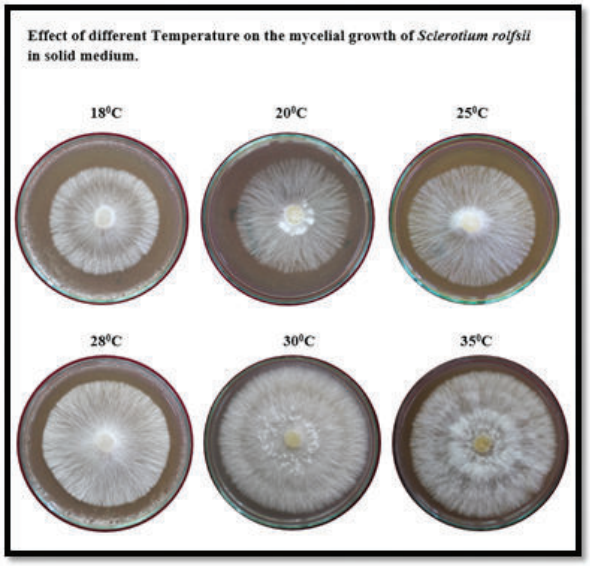

Fig. 8: Effect of different temperatures on the hyphal development of *S. rolfsii*

hydroxide. Sterilized PDA medium (20 ml) from each of the stock media with different pH levels was poured into Petri plates and the pathogens were inoculated on it.

The eight different isolates of Sclerotium were artificially inoculated on the tuberose plants in separate pots. All the eight isolates were found to exhibit pathogenicity but isolate SR1 recorded maximum per cent disease incidence (88.88%) followed by SR2. Hence, the SR1 isolate was found to be more virulent (Fig.. 1). Similarly, Sivakumar *et al*. (2016) used sorghum grains-based inoculum for the mass multiplication of *S. rolfsii* and proved the pathogenicity of groundnut collar rot and noticed maximum disease

incidence in isolate SR1 (55.7 %) and minimum disease incidence in SR5 (30.6 %).

In this study, all the eight different isolates grown in PDA medium were found to have variations in growth pattern (fluffy to profusely thick), colony morphology, hyphal colour (white to dull white colour), sclerotial colour (dark brown to reddish-brown), size of sclerotia (1.06 – 1.69 mm), the shape of sclerotia (round, irregular and spherical), sclerotial weight (70 - 180 mg /weight of 100 sclerotial bodies), sclerotial production duration (9 - 15 days) and arrangements (from the periphery to all over Petri plates) (Table 1) (Fig. 2).

Manu *et al*. (2018) also observed phenotypical characters of different isolates of *S. rolfsii* isolated from different crop cultivated areas of Southern Karnataka. They reported that the isolates produced colony with diameters ranging from 4.1 to 8.0 cm, several sclerotial bodies per plate from 261.7 to 1048.7, the sclerotial colour of light brown to dark brown, sclerotial size of 1.1 - 2.1 mm, the shape of round to spherical and weight of 4.4 -71.0 mg.

In present study, PDA medium recorded maximum fungal hyphal growth (9.00cm) followed by carrot dextrose agar medium (8.85cm) (Table 2, Fig. 3) which was similar to the earlier finding observed by Banakar and Sahana (2017) who noticed that maximum growth of *S. rolfsii* occurred in PDA medium after 48 hours of the incubation period.

In present study, among the ten different liquid broths tested, potato dextrose broth gave maximum fungal mycelial dry weight (7.30g) followed by carrot dextrose broth (6.26g) (Table 3) (Fig. 4). This finding correlated with the reports of Muthukumar and Venkatesh (2013) who noticed maximum hyphal dry weight of pathogen in potato dextrose broth.

Seven different carbon sources were tested in Czapek's dox agar medium in the present study. Starch as a carbon source in Czapek's dox agar medium recorded maximum mycelial growth (8.29cm) followed by dextrose (8.18cm) (Table 4) (Fig. 5). The results indicated that the basidiomycetous fungus produced degrading enzymes (sucrase and amylase) which might have utilized the carbon sources such as starch and sucrose efficiently.

Among the different nitrogen sources, maximal mycelial growth was recorded in sodium nitrate (8.77cm) followed by potassium nitrate (5.38cm) (Table 5) (Fig. 6). These results are coinciding with the results given by Sekhar *et al*. (2020) who confirmed that starch and dextrose were the best carbon sources for *Sclerotium* hyphal growth, whereas sodium nitrate and potassium nitrate were the best nitrogen sources.

In present study, maximal fungal hyphal growth of *Sclerotium* was found at pH 7.0 (8.92 cm) followed by pH 8.0 (7.89 cm) (Table 6) (Fig. 7). However, Muthukumar *et al*. (2013) reported the vigourous mycelial growth of *Sclerotium* at pH 6.0. Similarly, Sravani and Ram Chandra (2019) reported that pH 6.0, 7.0 and 8.0 has given maximum fungal growth.

In the present study, maximum fungal mycelial growth was observed at 30^0C (9.00 cm) followed by 35^0C (8.77 cm) (Table 7) (Fig. 8). Similarly, Sravani and Ram Chandra (2019) observed that hyphal development of *S. rolfsii* was maximum at 30^0C within five days followed by 20, 25 and 15^0C. The least mycelial growth was observed at 35^0.

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